Supplemental Material

The mechanics of single cross-links at the surface of PEGDA-co-ACPEG hydrogels

Arzu Çolak[1,‡,#], Bin Li[1,‡], Johanna Blass[1], Kaloian Koynov[2], Aranzazu del Campo[1], Roland Bennewitz[1,]*

[1] INM – Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbrücken, Germany

[2] Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

[#] current address: Department of Mechanical Engineering, University of California at Merced, Merced CA 95343, USA

[‡] These authors contributed equally to this work.

[*]Author to whom correspondence should be addressed (roland.bennewitz@leibniz-inm.de)

Control experiments on the specificity of the biotin-streptavidin bond

The length of the last rupture event is plotted as function of the rupture force for the last unbinding event in force curves recorded on biotin-functionalized 4 kPa gel in Figure S1 (black dots). In order to confirm the assignment of the unbinding events represented by black dots in Fig. S1 to specific streptavidin-biotin binding, the biotin molecules on hydrogels were passivated by immersing the hydrogel samples in streptavidin solution (0.25 mg/mL) for 30 min. Blocking the biotin in the hydrogel did not change its elastic modulus as confirmed by colloidal probe nanoindentation. We also confirmed that streptavidin diffused efficiently into the hydrogel by repeating the blocking experiment with fluorescently labeled streptavidin and observing

homogeneous distribution of fluorescence by confocal microscopy. The effect of blocking biotin interaction sites on the rupture forces was a reduction of the rupture length and rupture forces. The distinct separation of black dots (no blocking) from red circles (blocking) in Fig. S1 confirms that the specific bond was blocked by passivation with streptavidin. The force curves which were recorded after blocking the biotin still exhibited the characteristic non-linear shape, but a rupture at lower forces and shorter length.

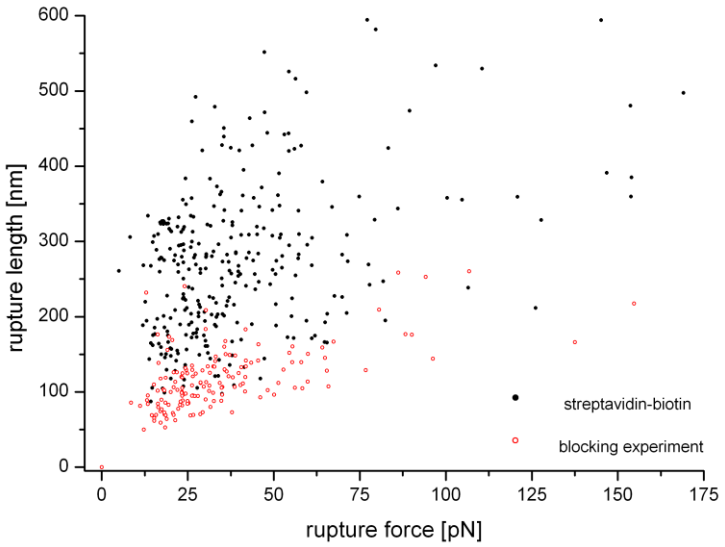

Figure S1. Rupture length as a function of rupture force for the 4 kPa biotinylated PEGDA-co-ACPEG hydrogel. Black dots represent the rupture events for the case when biotin at the hydrogel can bind to streptavidin at the tip. Red dots represent rupture events recorded after blocking the biotins at the hydrogel with streptavidin molecules.

Dependence of cell area on elastic modulus of substrate

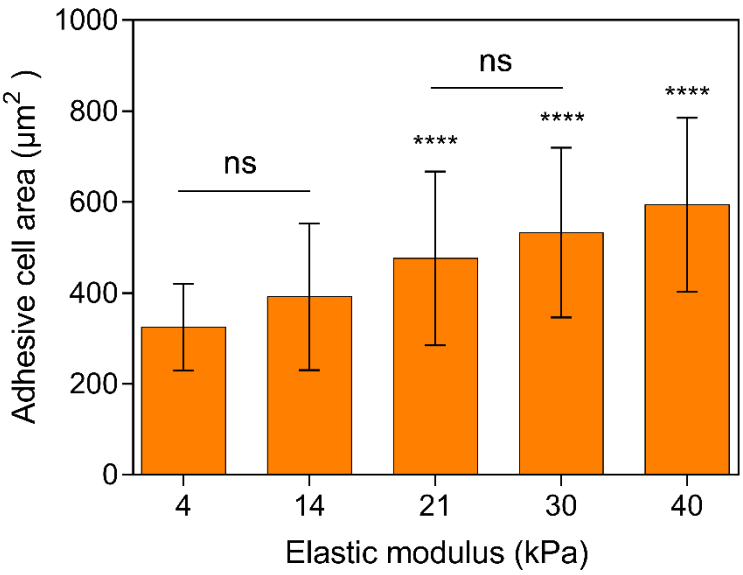

Figure S2. Quantified cell area of fibroblasts on RGD functionalized PEGDA-co-ACPEG hydrogels with varying elastic modulus. A significant increase in the cell area with increasing elastic modulus was observed. Orange boxes indicate the mean values, black lines the standard deviation. Significance differences when compared with the 4 kPa gel are indicated by *at a level $P < 0.0001$.